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Nitric Oxide Modulates Water and Electrolyte Transport in the Ileum

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Objective

To test the hypothesis that nitric oxide is a modulator of ileal water and ion transport.

Summary Background Data

Nitric oxide is produced in the vascular endothelium and enteric neural plexuses of the intestine and is involved in gastrointestinal motility and smooth muscle contractility. Little is known about the role of nitric oxide in intestinal epithelial transport.

Methods

Ten-centimeter rabbit ileal segments (n = 50) were vascularly perfused with an electrolyte solution containing red cells. The lumen was perfused with a solution containing ^{14}C-PEG. Net fluxes of water and ions were calculated during three 20-minute periods: basal, drug infusion, and recovery. Perfusion pressure was recorded to document changes in vascular resistance. Agents infused included the nitric oxide synthase substrate L-arginine, the nitric oxide source sodium nitroprusside, the substrate control D-arginine, and the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester.

Results

L-arginine and sodium nitroprusside caused absorption of water and ions. N^G-nitro-L-arginine methyl ester caused secretion of water and ions, which was prevented by synchronous infusion of L-arginine. Infusion of D-arginine had no effect. Both L-arginine and sodium nitroprusside caused mild vasodilation.

Conclusions

Inhibition of endogenous nitric oxide synthesis by N^G-nitro-L-arginine methyl ester causes secretion of water and ions. This secretion is reversed by administration of the nitric oxide synthase substrate L-arginine. These findings are consistent with the hypothesis that endogenous nitric oxide has a proabsorptive influence over the ileum in the basal state.

Endothelium-derived relaxing factor, a labile substance formed by endothelial cells, mediates vasodilation and is identical to nitric oxide.[1,2] In addition to its role in vasodilatation, nitric oxide is synthesized in many nonvascular tissues. In platelets, nitric oxide generation from L-arginine acts as a negative feedback on platelet aggregation.[3] In neutrophils and macrophages, nitric oxide acts as an effector molecule mediating cytotoxicity.[4] In neural tissues, nitric oxide is generated in response to activation of excitatory amino acid receptors, and it activates soluble guanylate cyclase in adjacent nerve terminals.[5] The enzyme responsible for nitric oxide synthesis from L-arginine is called nitric oxide synthase. It is convenient to consider the nitric oxide synthases as a family of enzymes, broadly grouped into two classes: constitutive and inducible.[6] Nitric oxide synthase is present con-

stitutively in the vascular endothelium, brain, platelets, adrenal gland, peripheral nerves, and other tissues, where it exists as a calmodulin-requiring enzyme.[7] Induction of nitric oxide synthase has been observed in vascular endothelium and smooth muscle, macrophages, neutrophils, Küppfer cells, hepatocytes, fibroblasts, and other cells.[6] These inducible nitric oxide synthases appear to be independent of calmodulin regulation.

In the guinea-pig small intestine, nitric oxide synthase immunoreactivity is present in myenteric neurons that have the projections and chemical coding of descending interneurons and enteric inhibitory motor neurons.[8,9] Nitric oxide synthase-immunoreactive axons make synapses on both nitric oxide synthase-immunoreactive and nonimmunoreactive myenteric neurons.[10] Evidence indicates that nitric oxide is an important neurotransmitter in the intestine, mediating nonadrenergic noncholinergic (NANC) inhibitory neurotransmission,[11-13] with resultant effects on gastrointestinal muscular contraction and motility.[14] In the gut, nitric oxide has also been implicated in gastrointestinal mucosal protection,[14,15] hemodynamic disturbances in cirrhosis,[14,16] and hepatocellular function and toxicity.[14,17] Nitric oxide's role in intestinal epithelial transport has been studied to a limited extent. Recently conflicting results showed that small bowel secretion is associated with increases in nitric oxide (produced by the nitric oxide synthase substrate L-arginine) and decreases in nitric oxide (produced by the selective nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester).[18,19] In our experiments, we tested the hypothesis that nitric oxide is a modulator of the transport of water and electrolytes in the mammalian ileum.

MATERIALS AND METHODS

The following protocol and methods were approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions. Fifty New Zealand white rabbits weighing 2.5 to 3.5 kg and maintained on standard laboratory food were fasted for 16 hours before study. We induced and maintained anesthesia with intravenous ketamine hydrochloride (Bristol Laboratories, Syracuse, NY). We infiltrated the midline abdominal wall with 1% lidocaine (Abbott Laboratories, North Chicago, IL) and isolated a 10-cm length of distal ileum proximal to the ileocecal attachment. We divided the bowel proximally and distally and cannulated the lumen with 2-cm-long silicone rubber tubing (inner diameter, 4 mm). We gently rinsed the bowel lumen with a warm buffered luminal perfusate solution (described below) until the luminal effluent was clear. We administered heparin sodium (Elkins-Sinn, Cherry Hill, NJ) intravenously in a dose of 100 U/kg of body weight, and we exposed and isolated the segmental mesenteric artery supplying the chosen intestinal segment. We cannulated the segmental mesenteric artery with polyethylene-90 tubing and flushed it with 0.9% heparin saline solution. The mesenteric vein was transected and allowed to drain freely. We removed the prepared, isolated distal ileal segment from the rabbits and transferred it to the perfusion apparatus, where we began vascular perfusion at a rate of 2 mL/min, yielding a mean arterial blood flow of 61.3 ± 1.4 mL/min per 100 g of tissue. Total warm ischemia time averaged 136 seconds ± 3 seconds. The intestinal temperature was maintained at 37 C with a heat lamp, and the bowel was covered to prevent desiccation. Arterial pressure was continuously measured with a water manometer located proximal to the arterial cannula.

The vascular and luminal perfusion circuits have been previously illustrated.[20] We oxygenated the vascular perfusate with a mixture of 95% O_2 and 5% CO_2 using a rotating-disk oxygenator. The vascular perfusate was pumped from the reservoir through a 40-μm filter (Ultipor Filter; Pall Corp., Glen Cove, NY) and a heat exchanger into the arterial cannula by a roller pump (Masterflex pump; Cole Palmer Instrument Co., Chicago, IL). We placed the entire perfusion system in a water bath (Model 210A; Napco, Portland, OR) maintained at 37 C. The vascular perfusate solution was prepared daily and consisted of a modified Krebs buffer solution containing washed human erythrocytes (hematocrit 15%), 3% bovine serum albumin (United States Biochemical Corp., Cleveland, OH), 25% dextran (Sigma Chemical Co., St. Louis, MO; molecular weight, 77,800), 5 mmol/L glucose, and 0.8 mmol/L glutamine. The final electrolyte concentrations of the vascular perfusate delivered to the isolated bowel were Na^+, 140 mEq/L; Cl^-, 100 mEq/L; K^+, 5.0 mEq/L; Ca^{2+}, 2.5 mEq/L; Mg^{2+}, 1.2 mEq/L; HPO_4^{2-}, 2.4 mEq/L; and HCO_3^-, 25 mEq/L. The pH of the perfusate was adjusted to 7.4 and the final osmolarity was 290 ± 5 m Osm/L.

We perfused the bowel lumen through the proximal cannula at 2 mL/min with a roller pump (Model 375A; Sage Instruments, Cambridge, MA). The luminal perfusate was collected by gravity from the distal luminal cannula with an automatic fraction collector (FRAC 100, Pharmacia Fine Chemicals, Uppsala, Sweden). The luminal perfusate was a buffered electrolyte solution that contained Na^+, 140 mEq/L; Cl^-, 120 mEq/L; K^+, 5.2

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mEq/L; Ca^{2+}, 1.2 mEq/L; Mg^{2+}, 1.2 mEq/L; HPO_4^{2-}, 2.4 mEq/L; $H_2PO_4^-$, 0.4 mEq/L; HCO_3^-, 25 mEq/L; glucose, 10 mmol/L and 10 μCi carbon 14-labeled polyethylene glycol (^{14}C-PEG) (New England Nuclear, Boston, MA) in 5 g/L of carrier PEG as a nonabsorbable marker.

Data Analysis

We analyzed the collected luminal effluent for electrolyte concentrations and ^{14}C-PEG activity. We determined Na^+ and Cl^- concentrations by ion-specific electrodes (Beckman E4A Analyzer, Irvine, CA). We assayed ^{14}C-PEG activity by liquid scintillation counting (Beckman Model LS 3801, Fullerton, CA). For each 20-minute interval (designated basal, drug infusion, and recovery), we calculated net water (measured in microliters per minute) and ion fluxes (measured in microequivalents per minute) from the change in ^{14}C-PEG activity and the luminal perfusion rate (2 mL/min). To calculate net water fluxes (F_{H2O}), we used the following equation[21]:

$$F_{H_2O} = 2\left[1 - \frac{[^{14}C]\text{-PEG in the infusate}}{[^{14}C]\text{-PEG in the effluent}}\right]$$

To calculate net ion fluxes (F_{ion}), we used this equation[21]:

$$F_{ion} = 2\left[[\text{Ion}]\text{infusate} - [\text{Ion}]\text{effluent}\left(\frac{[^{14}C]\text{-PEG infusate}}{[^{14}C]\text{-PEG effluent}}\right)\right]$$

To calculate recovery (R) of ^{14}C-PEG at 10-minute collection intervals, we used this equation[22]:

$$R = \frac{[^{14}C]\text{-PEG in the effluent} \times \text{effluent volume}}{[^{14}C]\text{-PEG in the infusate} \times \text{infusate volume}} \times 100$$

We used these recovery calculations to validate each collection period. We rejected recoveries not within 100% ± 5% and discarded data from these study periods. None of the study periods was discarded on the basis of recovery criteria. We calculated oxygen consumption using standard methods[23] at 20-minute intervals during the experiment. Statistical analyses were performed by analysis of variance and Student's t test, with significance accepted at the 5% level. Results are expressed as mean ± SEM.

Experiment Design

After the initiation of vascular and luminal perfusion of the *ex vivo* ileal segment, a 10-minute stabilization period was followed by a 60-minute experiment (Fig. 1). We divided each 60-minute experiment into three 20-minute periods: basal, drug infusion, and recovery. We studied eight groups in random order: (1) control (n = 6); (2) L-arginine infusion at 10^{-3} mol/L (n = 10); (3) L-arginine infusion at 10^{-2} mol/L (n = 6); (4) D-arginine at 10^{-3} mol/L (n = 4); (5) D-arginine infusion at 10^{-2} mol/L (n = 4); (6) sodium nitroprusside infusion at 10^{-4} mol/L (n = 8); (7) N^G-nitro-L-arginine methyl ester (L-NAME) infusion at 10^{-4} mol/L (n = 6); and (8) L-arginine infusion at 10^{-2} mol/L plus L-NAME infusion at 10^{-4} mol/L (n = 6). All agents were purchased from Sigma Chemical Company (St. Louis, MO) and were infused through a side arm in the arterial circuit at the rate of 0.025 mL/min by an infusion pump (Pump 22; Harvard Apparatus Co., South Natick, MA).

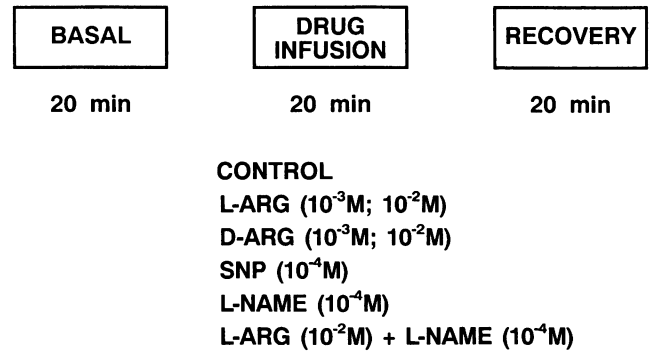

Figure 1. Schematic diagram of the experimental design.

RESULTS

All preparations of the isolated perfused rabbit ileum (n = 50) included in the data analyses were viable, normal-appearing preparations. We calculated the oxygen consumption of each ileal segment at 20-minute intervals and it was within the normal range for these preparations. The calculated oxygen consumption ranged from a mean of 0.7 ± 0.1 to 1.6 ± 0.2 mL O_2/min per 100-g wet weight of tissue for each of the experimental groups, with a mean of 1.01 ± 0.03 mL O_2/min per 100-g of wet weight for all measurements. None of the infused agents caused significant changes in the calculated oxygen consumption.

Water and Electrolyte Fluxes

The calculated fluxes of water, sodium, and chloride for each of the eight experimental groups followed over time are given in Table 1. To better allow for comparisons between the groups, the change in water and ion

Table 1. THE EFFECT OF L-ARG, D-ARG, SNP, AND L-NAME ON THE FLUXES OF WATER AND ELECTROLYTES

Group	Flux	Basal	Drug Infusion	Recovery
Control (n = 6)	H_2O	−30.8 ± 4.7	−33.5 ± 8.1	−33.7 ± 6.2
	Na^+	−5.1 ± 1.0	−5.8 ± 1.2	−5.6 ± 1.1
	Cl^-	−1.6 ± 0.4	−2.6 ± 0.9	−2.6 ± 0.6
L-ARG (10^{-3} mol/L) (n = 10)	H_2O	−22.9 ± 4.6	−15.3 ± 6.3	−38.1 ± 10.2*
	Na^+	−3.6 ± 0.7	−2.9 ± 1.1	−6.2 ± 1.5*
	Cl^-	−1.6 ± 0.6	−1.3 ± 0.8	−4.5 ± 1.3*
L-ARG (10^{-2} mol/L) (n = 6)	H_2O	−31.6 ± 6.8	−10.3 ± 5.9*	−27.4 ± 8.5
	Na^+	−4.4 ± 1.0	−1.9 ± 0.9*	−4.0 ± 1.2
	Cl^-	−2.7 ± 0.9	−1.0 ± 0.7*	−3.3 ± 1.0
D-ARG (10^{-3} mol/L) (n = 4)	H_2O	−24.3 ± 8.6	−29.9 ± 10.2	−41.4 ± 14.9
	Na^+	−4.7 ± 1.3	−4.9 ± 1.5	−7.0 ± 1.8
	Cl^-	−2.3 ± 1.2	−2.9 ± 1.2	−4.4 ± 1.1
D-ARG (10^{-2} mol/L) (n = 4)	H_2O	−27.0 ± 9.1	−28.0 ± 9.3	−34.5 ± 7.3
	Na^+	−4.4 ± 1.5	−3.8 ± 1.5	−5.3 ± 1.3
	Cl^-	−2.1 ± 1.3	−2.1 ± 1.1	−3.6 ± 0.8*
SNP (10^{-4} mol/L) (n = 8)	H_2O	−34.1 ± 5.9	−23.4 ± 7.6	−38.1 ± 10.5
	Na^+	−4.0 ± 1.1	−2.8 ± 1.0	−3.7 ± 1.3
	Cl^-	−3.1 ± 0.6	−2.1 ± 0.7	−3.9 ± 1.3
L-NAME (10^{-4} mol/L) (n = 6)	H_2O	−38.1 ± 8.5	−52.5 ± 4.1	−52.9 ± 9.1
	Na^+	−5.8 ± 1.2	−7.9 ± 0.7	−7.3 ± 1.3
	Cl^-	−3.6 ± 1.2	−6.1 ± 0.6*	−5.9 ± 1.0*
L-ARG (10^{-2} mol/L) + L-NAME (10^{-4} mol/L) (n = 6)	H_2O	−19.3 ± 4.1	−5.4 ± 5.9	−28.2 ± 15.8
	Na^+	−3.6 ± 1.0	−1.3 ± 1.3	−4.7 ± 2.6
	Cl^-	−1.5 ± 0.8	−0.3 ± 0.7	−3.4 ± 2.1

L-ARG = L-arginine; D-ARG = D-arginine; SNP = sodium nitroprusside; L-NAME = N^G-nitro-L-arginine methyl ester. H_2O flux in μL/min. Na^+ and Cl^- fluxes in μEq/min. Negative flux equals net secretion.

* $p < 0.05$ compared to Basal by ANOVA.

fluxes (ΔF) between the basal and drug infusion periods for each of the eight groups are shown in Figure 2.

We found a stable pattern of net water and electrolyte secretion during the 60-minute perfusion period in the control experiments, data that are consistent with our past observations.[20]

The infusion of L-arginine at 10^{-3} mol/L reduced the basal levels of water and electrolyte fluxes during the drug infusion period and significantly increased ($p < 0.05$) secretion during the recovery period. The infusion of L-arginine at 10^{-2} mol/L significantly decreased the net negative F_{H2O} from a basal level of -31.6 ± 6.8 μL/min to -10.3 ± 5.9 μL/min during the drug infusion period ($p < 0.05$), with a return to basal secretory levels during the recovery period. The magnitude of basal Na^+ and Cl^- secretion also decreased significantly during the 10^{-2} mol/L L-arginine infusion ($p < 0.05$), with a return to basal secretory levels during the recovery period. Thus L-arginine infusion at 10^{-2} mol/L (Fig. 3), which provides substrate for nitric oxide synthase, caused a significant proabsorptive effect on the transport of water and electrolytes.

We used D-arginine as an inactive substrate control for the L-arginine infusions. D-arginine cannot be converted to nitric oxide via nitric oxide synthase. Infusions of D-arginine at 10^{-3} mol/L and 10^{-2} mol/L did not affect transport of water or electrolytes. Sodium nitroprusside was used as a nitric oxide source. Infusion of sodium nitroprusside at 10^{-4} mol/L reduced the magnitude of basal water and electrolyte secretion during the drug-infusion period, with return toward basal values during the recovery period.

Infusion of the nitric oxide synthase inhibitor L-NAME at 10^{-4} mol/L increased the magnitude of secretion of water and electrolytes during the drug-infusion and recovery periods (Fig. 4). We observed significant increases in chloride flux ($p < 0.05$) during and after the L-NAME infusion. Thus, in direct contrast to the proabsorptive effects on water and electrolyte transport observed with L-arginine (substrate for nitric oxide synthase) and sodium nitroprusside (nitric oxide source), L-NAME was associated with increased secretion of water and electrolytes in the isolated ileum.

The combined infusion of L-NAME at 10^{-4} mol/L and an excess of the nitric oxide synthase substrate L-arginine at 10^{-2} mol/L comprised the last group studied.

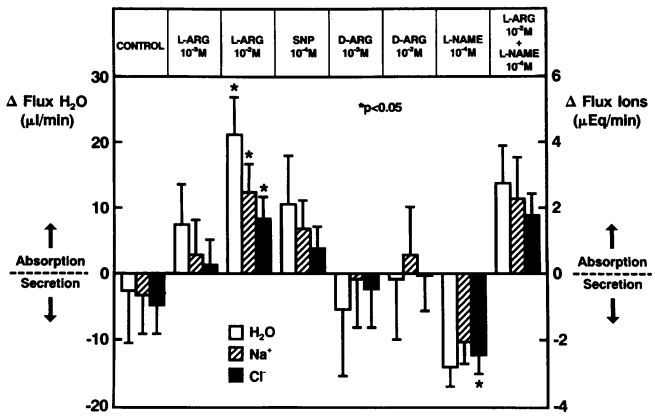

Figure 2. The effects of L-arginine (L-ARG), sodium nitroprusside (SNP), D-arginine (D-ARG), and N^G-nitro-L-arginine methyl ester (L-NAME) on the change in water and electrolyte flux between the basal and drug infusion periods. Positive ΔFlux indicates a proabsorptive effect. Negative ΔFlux indicates a prosecretory effect. Both L-ARG and SNP caused proabsorptive effects, significant at 10^{-2} mol/L L-ARG for H_2O, Na^+, and Cl^-. L-NAME caused a prosecretory effect, significant for Cl^-. The L-NAME-induced prosecretory effect was reversed by providing excess nitric oxide synthase substrate L-ARG.

Compared with the infusion of L-NAME alone at 10^{-4} mol/L, the addition of L-arginine at 10^{-2} mol/L (Fig. 2) reversed the prosecretory effect of L-NAME alone, to yield a proabsorptive effect similar to the actions of L-arginine alone.

Perfusion Pressure

The changes in perfusion pressure recorded during these 60-minute experiments averaged +24 ± 4 cm H_2O in the control group, +22 ± 3 cm H_2O in the 10^{-3} mol/L L-arginine group, +17 ± 3 cm H_2O in the 10^{-2} mol/L L-arginine group, +17 ± 10 cm H_2O in the 10^{-3} mol/L D-arginine group, +14 ± 4 cm H_2O in the 10^{-2} mol/L D-arginine group, +9 ± 4 cm H_2O in the sodium nitroprusside group, +38 ± 8 cm H_2O in the L-NAME group, and +24 ± 3 cm H_2O in the L-arginine + L-NAME group. These changes in perfusion pressure in the control group are similar to our previous observations in control experiments.[20] We observed the smallest change in perfusion pressure in the sodium nitroprusside group, a known vasodilator and nitric oxide source. The largest change in perfusion pressure occurred in the L-NAME group, an inhibitor of nitric oxide synthase.

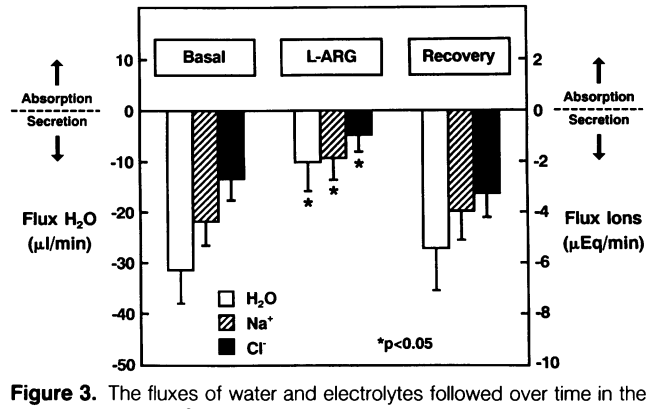

Figure 3. The fluxes of water and electrolytes followed over time in the group receiving 10^{-2} mol/L L-arginine (L-ARG; n = 6). Negative flux indicates secretion. The infusion of L-ARG caused a significant reduction ($p < 0.05$) of basal secretion of water, sodium, and chloride during the drug infusion period, indicating a proabsorptive effect.

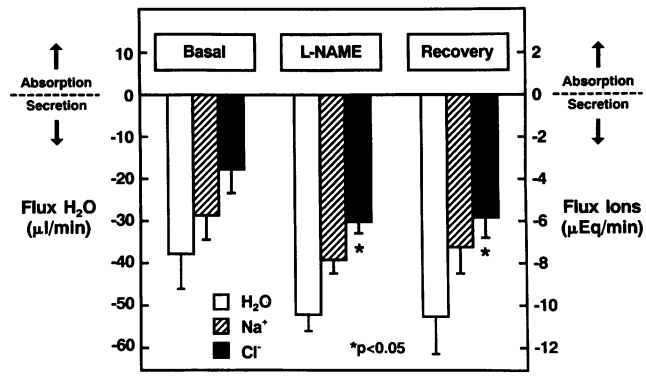

Figure 4. The fluxes of water and electrolytes followed over time in the group receiving 10^{-4} mol/L N^G-nitro-L-arginine methyl ester (L-NAME; n = 6). Negative flux indicates secretion. The infusion of L-NAME caused increased secretion of water and sodium and significantly increased ($p < 0.05$) secretion of chloride during the drug infusion and recovery periods, indicating a prosecretory effect.

DISCUSSION

The transport of water, electrolytes, and nutritive substances by the small intestine maintains homeostasis and fulfills a nutritive role. The net transport state of the intestine depends on a summation of factors, including the composition of luminal contents, neural inputs from extraintestinal anatomic pathways and intrinsic enteric pathways, effects of circulating hormones with activity on epithelial transport, and the effects of these stimuli on bowel motility and intestinal blood flow. We used an *ex vivo*, vascularly perfused rabbit ileal preparation to determine the effect of nitric oxide on ileal transport of water and electrolytes. This preparation has several advantages over other techniques used to study intestinal transport and has been validated and used extensively to study intestinal transport.[20,24–28] It is a full-thickness intestinal preparation without intestinal wall stripping or destruction. The preparation contains a locally intact enteric nervous system but is devoid of higher extraintestinal anatomic innervation. Strict control of intestinal blood flow is maintained, thereby avoiding confounding influences from alterations in blood flow. We used a non-

recirculating arterial perfusate, eliminating the possibility of recirculating agents that might interfere with the absorption or secretion characteristics of the intestine. Finally, the calculation of ^{14}C-PEG recovery from the luminal effluent serves as an indirect control for intestinal motility effects, because agents that increase motility would be associated with increased ^{14}C-PEG recovery and agents that decrease motility would be associated with luminal pooling and decreased ^{14}C-PEG recovery.

In mammals, the importance of an inhibitory component in intestinal motility was recognized when Bayliss and Starling[29] demonstrated intrinsic neural reflexes nearly 100 years ago. Until recently, the identity of the NANC inhibitory neurotransmitter was unknown. Current evidence has implicated nitric oxide as the mediator of NANC inhibition, based on data showing (1) nitric oxide release from the stomach and ileocolonic junction during nerve stimulation,[30,31] (2) that exogenous nitric oxide mimics NANC nerve-evoked relaxation,[32,33] (3) that inhibition of nitric oxide synthesis interferes with NANC inhibition,[34] and (4) localization of nitric oxide synthase to the myenteric plexus and intestinal nerves. In addition to its role in NANC inhibition of intestinal motility, nitric oxide has been implicated in the regulation of gastric mucosal microcirculation[15,35] and pancreatic blood flow and exocrine secretion.[36] We began our studies to evaluate the role of nitric oxide as a mediator of small intestinal transport.

Our results suggest that the endogenous production of nitric oxide exerts a tonic proabsorptive effect on the transport state of the ileum. Support for this comes from the observations (Fig. 2) that (1) infusions of both L-arginine and sodium nitroprusside are associated with proabsorptive effects for water and electrolytes, (2) infusion of L-NAME is associated with a prosecretory response for water and electrolytes, and (3) infusion of L-arginine during simultaneous L-NAME infusion results in a proabsorptive response for water and electrolytes. These observations are internally consistent, and are further validated by the observation that infusion of D-arginine, an enantiomer of L-arginine that is not a substrate for nitric oxide synthase, has no transport effects.

Circulating hormones and local neurotransmitters play an important role in the control of intestinal water and electrolyte transport.[37,38] Previous studies indicated that norepinephrine,[24,25] somatostatin,[27] and neuropeptide Y[28] increase ileal water and ion absorption at a similar magnitude to that observed with L-arginine and sodium nitroprusside. The intracellular second messenger systems responsible for these proabsorptive responses have not been fully elucidated. However evidence supports activation of protein kinase C and inhibition of adenylate cyclase[26,39] as probable pathways of signal transduction. The involvement of nitric oxide as an endogenous mediator of intestinal transport further implicates the guanylate cyclase second messenger system, because many studies have documented that increases in nitric oxide production are followed by binding of nitric oxide to soluble guanylate cyclase, a cytosolic heme-containing enzyme that increases the production of 5′-cyclic guanosine monophosphate.[2,5]

In our experiments using the *ex vivo* perfused ileum, we did not directly measure intestinal motility. However, we made an indirect assessment of the luminal area and contractile state by monitoring recovery of the nonabsorbable marker ^{14}C-PEG in the luminal perfusate solution over time. The recovery of luminal ^{14}C-PEG remained at 100% ± 5% of the delivered ^{14}C-PEG throughout the experiments, suggesting that changes in motility, intestinal diameter, or mucosal surface area were not primarily responsible for the proabsorptive effects seen with L-arginine and sodium nitroprusside. In addition, we fixed the total intestinal blood flow at a constant rate, thereby eliminating alterations in total intestinal blood flow as a cause of the changes in intestinal transport. Thus in these experiments, direct involvement of nitric oxide in intestinal transport is strongly supported. Further support for a direct effect of nitric oxide on intestinal transport is provided by measurement of the end products of nitric oxide formation or by assay of 5′-cyclic guanosine monophosphate levels and guanylate cyclase activity at the enterocyte level.

Observations made during luminal infusion of L-arginine and L-NAME to rat jejunum[19] and canine ileum (our unpublished observations) lend additional support to the theory that nitric oxide influences intestinal transport at the enterocyte level. Mourad and colleagues[19] observed that inhibition of nitric oxide synthesis with luminal L-NAME (20 mmol/L) led to jejunal water secretion, which was attenuated by luminal L-arginine (20 mmol/L). Similarly, using a conscious canine Thiry-Vella fistula model, we observed that luminal L-NAME (10 mmol/L) reduces basal ileal water and ion absorption, while luminal L-arginine (10 mmol/L) stimulates a proabsorptive response.

Our experiments showed a proabsorptive effect of exogenously administered L-arginine and sodium nitroprusside on the ileal transport of water and electrolytes. Furthermore, we found a prosecretory effect with exogenous administration of the nitric oxide synthase inhibitor L-NAME. These data are consistent with the hypothesis that endogenous nitric oxide serves as a physiologic proabsorptive modulator of ileal water and electrolyte transport.

References

1. Ignarro LJ. Endothelium-derived nitric oxide: actions and properties. FASEB J 1989; 3:31–36.

2. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 1991; 43: 109–142.
3. Radomski MW, Palmer RMJ, Moncada S. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. Proc Natl Acad Sci USA 1990; 87:5193–5197.
4. Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. Biochem Biophys Res Commun 1988; 157:87–94.
5. Bredt DS, Snyder SH. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proc Natl Acad Sci USA 1989; 86:9030–9033.
6. Palmer RMJ. The discovery of nitric oxide in the vessel wall. A unifying concept in the pathogenesis of sepsis. Am J Surg 1993; 128:396–401.
7. Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci USA 1990; 87: 682–685.
8. Costa M, Furness JB, Brookes SJH, Bredt DS, Snyder SH. Presence and chemical coding of neurons with nitric oxide synthase immunoreactivity in the guinea-pig small intestine. Proc Aust Physiol Pharmacol Soc 1991; 22:97P.
9. Furness JB, Costa M, Pompolo S, et al. Projections of neurons with nitric oxide synthase immunoreactivity in the guinea-pig small intestine. Proc Aust Physiol Pharmacol Soc 1991; 22:99P.
10. Llewellyn-Smith IJ, Song Z-M, Costa M, Bredt DS, Snyder SH. Ultrastructural localization of nitric oxide synthase immunoreactivity in guinea-pig enteric neurons. Brain Res 1992; 577:337–342.
11. Dalziel HH, Thornbury KD, Ward SM, Sanders KM. Involvement of nitric oxide synthetic pathway in inhibitory junction potentials in canine proximal colon. Am J Physiol 1991; 260:G789–G792.
12. Murray JC, Du C, Ledlow A, Bates JN, Conklin JL. Nitric oxide: mediator of nonadrenergic noncholinergic responses of opossum esophageal muscle. Am J Physiol 1991; 261:G401–G406.
13. Shuttleworth CWR, Murphy R, Furness JB. Evidence that nitric oxide participates in non-adrenergic inhibitory transmission to intestinal muscle in the guinea-pig. Neurosci Lett 1991; 130:77–80.
14. Stark ME, Szurszewki JH. Role of nitric oxide in gastrointestinal and hepatic function and disease. Gastroenterology 1992; 103: 1928–1949.
15. Pique JM, Whittle BJR, Esplugues JV. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. Eur J Pharmacol 1989; 174:293–296.
16. Claria J, Jimenez W, Ros J, et al. Pathogenesis of arterial hypotension in cirrhotic rats with ascites: role of endogenous nitric oxide. Hepatology 1992; 15:343–349.
17. Billiar TR, Curran RD, Stuehr DJ, et al. An L-arginine-dependent mechanism mediates Kupffer cell inhibition of hepatocyte protein synthesis in vitro. J Exp Med 1989; 169:1467–1472.
18. Mourad FH, O'Donnell LJD, Clark ML, Farthing MJG. L-arginine induces water secretion in rat jejunum in vivo. Gastroenterology 1993; 104:A266.
19. Mourad FH, O'Donnell LJD, Clark ML, Farthing MJG. Effect of nitric oxide synthase inhibition on water absorption in rat jejunum. Gastroenterology 1993; 104:A266.
20. Yeo CJ, Couse NF, Zinner MJ. Serotonin and substance P stimulate intestinal secretion in the isolated perfused ileum. Surgery 1989; 105:86–92.
21. Bright-Asare P, Binder HJ. Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. Gastroenterology 1973; 64:81–88.
22. Miller DL, Schedl HP. Total recovery studies of nonabsorbable indicators in the rat small intestine. Gastroenterology 1970; 58:40–46.
23. Kvietys PR, Granger DN. Relation between intestinal blood flow and oxygen uptake. Am J Physiol 1982; 242:G202–G208.
24. Yeo CJ, Couse NF, Antiohos C, Zinner MJ. The effect of norepinephrine on intestinal transport and perfusion pressure in the isolated perfused rabbit ileum. J Surg Res 1988; 44:617–624.
25. Yeo CJ, Couse NF, Zinner MJ. Discrimination between $alpha_1$ and $alpha_2$ adrenergic receptors in the isolated perfused ileum. Surgery 1988; 104:130–136.
26. Yeo CJ, Bastidas JA, Schmieg RE Jr, et al. Post-receptor mechanisms of small bowel water and electrolyte transport. Surgery 1989; 106:408–415.
27. Anthone GJ, Bastidas JA, Orandle MS, Yeo CJ. Direct proabsorptive effect of octreotide on ionic transport in the small intestine. Surgery 1990; 108:1136–1142.
28. Anthone GJ, Orandle MS, Wang BH, Yeo CJ. Neuropeptide Y-induced intestinal absorption: mediation by α_2-adrenergic receptors. Surgery 1991; 110:1132–1138.
29. Bayliss WM, Starling EH. The movements and innervation of the small intestine. J Physiol (London) 1899; 24:99–143.
30. Boeckxstaens GE, Pelckmans PA, Bogers JJ, et al. Release of nitric oxide upon stimulation of nonadrenergic noncholinergic nerves in the rat gastric fundus. J Pharmacol Exp Ther 1991; 256:441–447.
31. Bult H, Boeckxstaens GE, Pelckmans PA, et al. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. Nature 1990; 345:346–347.
32. Ward SM, McKeen ES, Sanders KM. Role of nitric oxide in non-adrenergic, non-cholinergic inhibitory junction potentials in canine ileocolonic sphincter. Br J Pharmacol 1992; 105:776–782.
33. Boeckxstaens GE, Pelckmans PA, Bult H, et al. Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. Eur J Pharmacol 1990; 190:239–246.
34. Stark ME, Bauer AJ, Sarr MG, Szurszewski JH. Nitric oxide mediates inhibitory nerve input in human and canine jejunum. Gastroenterology 1993; 104:398–409.
35. Pique JM, Esplugues JV, Whittle BJR. Endogenous nitric oxide as a mediator of gastric mucosal vasodilatation during acid secretion. Gastroenterology 1992; 102:168–174.
36. Konturek SJ, Bilski J, Konturek PK, Cieszkowski M, Pawlik W. Role of endogenous nitric oxide in the control of canine pancreatic secretion and blood flow. Gastroenterology 1993; 104:896–902.
37. Levin RJ. The effects of hormones on the absorptive, metabolic and digestive functions of the small intestine. J Endocrinol 1969; 45:315–348.
38. Hubel KA. Intestinal nerves and ion transport: stimuli, reflexes and responses. Am J Physiol 1985; 248:G261–G271.
39. Bastidas JA, Zinner MJ, Orandle MS, Yeo CJ. Neural blockade in basal and postreceptor-stimulated intestinal transport. J Surg Res 1990; 48:427–434.